Effect of dietary protein and protein energy ratio on the growth performance of lemon fin barb hybrid (*Hypsibarbus wetmorei* × *Puntius gonionotus*) larvae

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Abstract

A series of two experiments was conducted to study the optimum dietary protein level and protein to energy ratio of lemon fin barb hybrid larvae. In Experiment 1, five isocaloric diets (4700 kcal per kg) ranging from 40 to 60% protein in 5% increments were fed to triplicate groups of lemon fin barb hybrid larvae (initial weight: 0.10 ± 0.01 mg per fish) for 21 days. Weight gain of fish was proportional to the protein content of the diet up to an incorporation rate of 50%. Among formulated diets, the diet with 50% protein produced the highest weight gain (4.26 ± 0.03 mg). The dietary protein level that yielded maximum growth was 52.1% based on a broken-line model estimation of weight gain. Based on these results, two dietary protein levels (50% and 55%) were used along with three energy levels (4500, 4700 and 4900 kcal per kg diet) at each protein level in Experiment 2. The survival of fish fed diets containing 55% protein was significantly lower ($p<0.05$) than that of fish fed diets containing 50% protein regardless of the energy level. Weight gain of fish was significantly ($p<0.05$) different at all dietary protein and energy levels. The best growth was observed in larvae fed diet 3 containing 50% protein with 4900 kcal/kg energy with the highest weight gain (3.12 ± 0.00 mg), feed conversion ratio (0.87 ± 0.02) and protein energy ratio (97.35 mg/kcal).

Keywords: Protein, Energy, Lemon fin barb hybrid, Larvae

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Introduction

Lemon fin barb hybrid is a new hybrid and a potential candidate for intensive aquaculture because of its desirable taste, hardiness in a crowded environment and rapid growth. It has successfully been produced by the cross breeding male Kerai Kunyit, *Hypsibarbus wetmorei* and female Lampam Jawa, *Puntius gonionotus*. Moreover it could be commercially produced in the form of salted fish, smoked fish and fermented fish. This fish can be cultured in urban areas such as in concrete tanks, canvas and can also be cultivated together with mixed vegetables such as in a pisciponic system. The market price for this species was around RM25 to RM48 per kilogram (Hatta, personal communication).

Most fish culturists do not know exactly the characteristics and requirement of this hybrid especially the protein energy ratio in feeds for this hybrid. According to Shiau and Lan (1996) and Bahnasawy (2009), protein is the most expensive part in fish feed. Thus, knowledge of the protein requirement is vital in formulating well-balanced and low cost feeds. It is crucial to determine protein requirements accurately for each species and for each size of cultured fish. To create an optimum diet, the ratio of protein to energy must be determined separately for each species. An optimum protein energy ratio will result in better growth and less cost for production of fish. Extra dietary protein will be utilized for energy and this led to ammonia excretion (Craig and Helfrich, 2009). Moreover, when inadequate dietary energy is fed, protein will not be used for growth but it will be used for energy. Therefore, balancing protein to energy in feed is crucial (Catacutan and Coloso, 1995). Due to the reasons, this research was conducted to study the optimum dietary protein to energy ratio of lemon fin barb hybrid larvae.

The optimum Protein/Energy (P/E) ratio (mg protein/kcal) has been determined for diets of several aquaculture species: larval silver barb (Tayag, 2004); juvenile Asean seabass (Catacutan and Coloso, 1995); juvenile green abalone (Gomez-Montes *et al.*, 2003) and grouper (Shiau and Lan, 1996). Here we report the optimum P/E ratio in diets for larval lemon fin barb hybrid.

Energy is a crucial part of diets and is considered as a basal component of food and in order to maintain body growth, energy is required. However, the most important part of the diet that acts as the main cost is protein (Ghiasvand *et al*., 2012). In order to reach maximum growth, abalone protein deposition must be maximized and formulated diets must comprise a proper balance of appropriate sources of protein and energy (Gomez-Montes *et al*., 2003). Energy and protein dietary nutrients are vital for the building of living tissues. They could be a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes (Craig and Helfrich., 2009).

Development and manufacturing of artificial diets that may replace natural feeds resulted from increased understanding of the nutritional requirements for fish species and technological advances in feed manufacturing (Abowei and Ekubo, 2011).

In aquaculture especially something that involves semi-intensive systems, artificial feeds should serve two functions which are to be promptly eaten by fish and the second is to provide nutrients to the environment that flourishes natural food availability (Rahman, 2006).

D'abramo (2002) reported that the industry of culturing larvae of many species of fish and crustaceans depends too much on live food. Even though *Artemia* and rotifers could serve as excellent sources, users should realize that live *Artemia* nauplii are obtained through cysts that are collected from the natural environment and this may be subject to periodic, unpredictable shortages that cannot always supply the demand. The effect of this phenomenon is increment in prices leading to increment in production costs. Temporal differences in cyst collections resulted in variation in the nutritional quality of *Artemia* and this was another problem that arose. In order to increase efficiency of larval fish production and eliminate too much dependance on live diets, improved diet formulation and manufacturing technologies should be foreseen (Barrows and Lellis, 2006).

The aim of this research was to appraise the specific nutritional requirements of lemon fin barb hybrid larvae which are prerequisites to ameliorate economical and productive potential of this freshwater fish.

Materials and methods

Preparation of microbound diet

Diet ingredients were selected following Tayag (2004) who studied larval nutrition of silver barb *Barbodes gonionotus* with an emphasis on protein-energy requirements. Fishmeal, casein, corn meal and rice bran were used as dietary protein, lipid and carbohydrate sources, respectively. Five gram of carrageenan (binder) was added to one hundred gram mixed formulated ingredients as shown in Tables 1 and 2 (finely ground) in a glass beaker. Then twenty five ml of water was added again and again until the mixture was mixed well and became sticky. Then the mixture was heated in 85˚C water bath. It was continuously stirred until the binder melted (smooth). The mixture was poured into an aluminum foil tray. It was cooled at 4˚C. The pudding was cut into cubes and the cubes were freeze dried for three days. The cubes were then ground and sieved with 50 µm sieve in the first day (for ration day 1 till day 9) and with 100 µm sieve in day 10 onwards (ration for subsequent rearing period).

Protein and protein energy requirement

Two-day old lemon fin barb hybrid larvae (initial weight: 0.10±0.01 mg per fish) were obtained from a single batch of artificially spawned breeders at the Aquaculture Extension Research Centre Perlok, Pahang. Larvae were randomly stocked into six (Experiment 1) and seven (Experiment 2) 10 L glass aquaria filled with 10 liters of fresh water, respectively. Larvae were stocked at a rate of 10 larvae L^{-1}.

Table 1: Formulation of proximate composition of experimental diets (% dry wt.) in Experiment 1. For control, crushed *Artemia* at the rate of approximately 5000 cells/L of water was given to larvae.

Diet code: Protein (%)/ Energy (kcal/kg)	1 50/4500	2 50/4700	3 50/4900	4 55/4500	5 55/4700
Ingredient					
Fish meal	10	10	10	10	10
Casein	34.25	40.55	47.90	55.80	62.61
Corn meal	7.91	0.00	0.00	4.09	0.00
Rice bran	31.18	34.53	28.13	16.68	15.32
Carrageenan	5	5	5	5	5
Fish oil	9.66	7.93	6.97	6.43	5.07
Mineral premix	1	1	1	1	1
Vitamin premix	1	1	1	1	1
Proximate composition (% dry wt.)					
Crude protein (%)	39.38	44.71	49.26	55.46	60.10
Ether extract	13.31	12.33	12.06	10.43	11.55
Ash	8.02	7.23	6.94	6.79	6.58
Crude fiber	1.22	1.15	0.88	0.98	1.21
Gross energy (kcal per kg)	4689.3	4701.2	4693.3	4704.4	4706.3

Table 2: Formulation and proximate composition of experimental diets (% dry wt.) in Experiment 2. For control, crushed *Artemia* at the rate of approximately 5000 cells/L of water was given to larvae.

Diet code: Protein (%)/ Energy (kcal/kg)	1 50/4500	2 50/4700	3 50/4900	4 55/4500	5 55/4700	6 55/4900
Ingredient						
Fish meal	10	10	10	10	10	10
Casein	47.42	47.90	48.87	54.32	55.69	56.31
Corn meal	3.65	0	0	0.22	3.23	0.62
Rice bran	28.58	28.13	23.17	26.42	17.75	16.00
Carrageenan	5	5	5	5	5	5
Fish oil	3.34	6.97	10.96	2.05	6.34	10.07
Mineral premix	1	1	1	1	1	1
Vitamin premix	1	1	1	1	1	1
Proximate composition (% dry wt.)						
Crude protein (%)	49.92	49.49	50.39	55.13	54.88	55.15
Ether extract	9.51	8.66	11.21	12.54	11.87	13.01
Ash	7.88	7.64	6.55	7.02	6.21	6.31
Crude fiber	1.67	1.32	0.76	0.99	1.05	0.74
Nitrogen-free extract	31.02	32.89	31.09	24.32	25.99	24.79
Gross energy (kcal per kg)	4488.53	4694.07	4906.79	4495.70	4689.29	4897.23
Protein/energy ratio(mg/kcal)	89.93	94.86	97.35	81.56	85.44	88.79

Five isocaloric diets with five varying protein levels (40%, 45%, 50%, 55% and 60%) and with energy level of 4700 kcal/kg (Tayag, 2004) were given to each experimental unit with three replicates in Experiment 1 (Refer to Table 1). The dietary protein level that yielded maximum growth was 52.1% based on regression analysis (dietary protein level versus weight gain). Based on this result, two dietary protein levels (50% and 55%) were used along with three energy levels (4500, 4700 and 4900 kcal per kg diet) at each protein level in Experiment 2. Six experimental diets were formulated (Refer to Table 2). Artificial diets were given at a rate of 45% of larval body weight with 3% increment daily at four feeding times; 0900, 1200, 1400 and 1700h (Tayag *et al*., 2005). For the control, larvae were solely given crushed *Artemia* for the whole rearing period at four feeding times; 0900, 1200, 1400 and 1700h. The larvae were fed the test diets for 21 day. Subsequent body weight measurements were taken from the representative samples of 10 larvae aquarium^{-1} at 4-day intervals from the start until the termination of the feeding experiment. Weight was determined using an analytical balance (Sartorius BT2245). Mortality was recorded daily and final survival was determined at the end of the experiment

Water quality management

Experimental aquaria were cleaned daily by siphoning unconsumed feed and fecal material. Ten percent of the total water volume was exchanged at 4-day intervals. Water quality parameters such as ammonia nitrogen, dissolved oxygen (D.O) and pH were monitored every 4 days while water temperature was monitored daily. The ranges of water quality parameters recorded are temperature 27-27.5˚C, pH 8.5-8.9, Dissolved Oxygen 5.7-5.8 mg/L, total alkalinity 68-80 ppm (mg/L) and ammonia nitrogen from 0.25-0.34 ppm. Dissolved oxygen and temperature were measured using a digital meter.

Statistical analysis

The variation in weight gain (mg) and survival of the larvae under different treatments were tested using one way analysis of variance (ANOVA). Significant results ($P<0.05$) were further tested using DMRT (Duncan Multiple Range Test) to identify significant differences among means. The statistical analysis was performed with the aid of computer software SPSS version 11.5 program.

Growth performance of the fish was calculated as described by Ahmad *et al.* (2012) as follows:

Weight gain (WG): final weight (mg)-initial weight (mg)

Food Conversion Ratio (FCR): diet fed (mg) / total wet weight gain (mg)

Average daily weight Gain (ADG): weight gain (mg)/ time (days)

Specific growth rate (SGR): 100(Ln final weight (mg) – Ln initial weight (mg))/ time (days)

Results

The growth characteristics of fish fed the the different dietary protein levels in Experiment 1 are presented in Table 3.

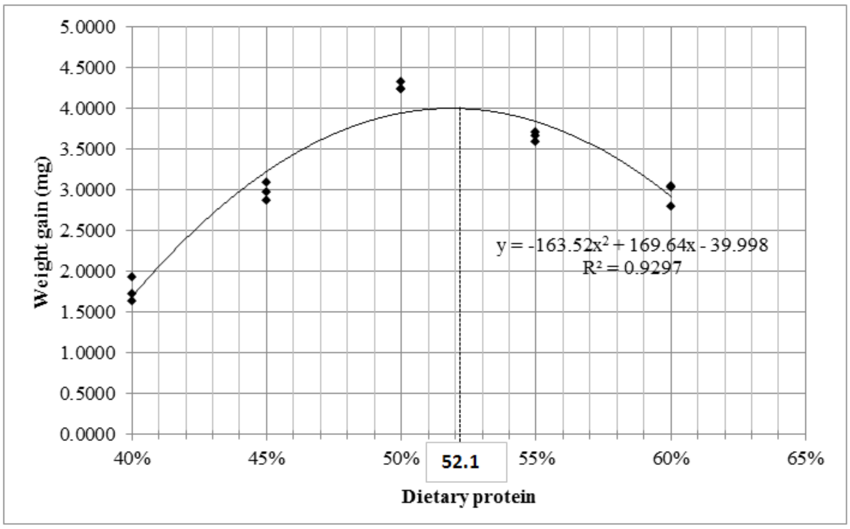

Figure 1: Broken-line analysis of the weight gain data detected a break point in the regression analysis (dietary protein level versus weight gain).

Table 3: Weight gain, survival, feed conversion ratio (FCR), average daily weight gain (ADG) and specific growth rate (SGR) of lemon fin barb larvae after 21 days of feeding with crushed *Artemia* (control) and the test diets containing five protein levels with 4700 kcal per kg energy.

Diet	Survival (%)	Weight Gain (mg)	Average Daily Weight Gain (mg/day)	Specific Growth Rate (%/day)	Feed Conversion Ratio
Control	76.00 ± 1.53^{a}	4.87 ± 0.06^{a}	0.30 ± 0.00^{a}	18.80 ± 0.26^{a}	*
40% protein	59.00 ± 6.03^{bc}	1.76 ± 0.09^{e}	0.11 ± 0.01^{e}	13.08 ± 0.72^{c}	1.67 ± 0.05^{a}
45% protein	64.33 ± 2.73^{b}	2.98 ± 0.06^{d}	0.19 ± 0.00^{d}	15.90 ± 1.01^{b}	1.27 ± 0.22^{ab}
50% protein	63.00 ± 2.89^{b}	4.26 ± 0.03^{b}	0.27 ± 0.00^{b}	18.46 ± 0.87^{ab}	0.62 ± 0.09^{c}
55% protein	52.00 ± 1.00^{c}	3.65 ± 0.03^{c}	0.23 ± 0.00^{c}	17.26 ± 0.97^{ab}	0.64 ± 0.12^{c}
60% protein	61.33 ± 2.96^{bc}	2.95 ± 0.08^{d}	0.18 ± 0.01^{d}	15.86 ± 1.00^{b}	0.85 ± 0.15^{bc}

Values are means±S.E.M. of three groups per treatment. Values in the same row with different superscript are significantly different (p<0.05).
* FCR for control was not calculated.

Weight gain was highest for fish fed *Artemia* (4.87±0.06 mg) in Experiment 1. Among formulated diets, larvae fed the diet containing 50% protein gained the highest weight gain (4.26±0.03 mg) and was significantly different (p<0.05) from other treatments including the control group. Broken-line analysis of the weight gain data detected a break point in the regression analysis (dietary protein level versus weight gain) when the dietary protein was 52.1%, suggesting that this level of protein was optimum for the growth of larval lemon fin barb hybrid. Due to this result, two dietary protein levels (50% and 55%) were used along with three energy levels (4500, 4700 and 4900 kcal per kg diet) at each protein level in Experiment 2.

Table 4: Weight gain, survival, feed conversion ratio (FCR), average daily weight gain (ADG) and specific growth rate (SGR) of lemon fin barb larvae after 21 days of feeding with crushed *Artemia* (control) and the test diets containing two protein levels with various energy contents.

	Energy (kcal per kg diet)				
	4500 (kcal/kg)	4700 (kcal/kg)	4900 (kcal/kg)	control	Group
Survival (%)					
50% protein	60.33 ± 0.88^{a}	56.33 ± 2.96^{b}	62.00 ± 2.08^{a}	66.67 ± 0.67	59.60 ± 1.37
55% protein	52.33 ± 2.19^{a}	55.00 ± 3.21^{a}	41.00 ± 1.53^{b}		49.40 ± 2.46
Weight gain (mg)					
50% protein	2.65 ± 0.01^{b}	2.09 ± 0.00^{c}	3.12 ± 0.00^{a}	4.46 ± 0.07	2.62 ± 0.15
55% protein	2.32 ± 0.00^{a}	1.85 ± 0.00^{c}	1.94 ± 0.01^{b}		2.04 ± 0.07
Average daily weight gain (mg/day)					
50% protein	0.17 ± 0.00^{b}	0.13 ± 0.00^{c}	0.20 ± 0.00^{a}	0.28 ± 0.00	0.16 ± 0.01
55% protein	0.15 ± 0.00^{a}	0.12 ± 0.00^{c}	0.12 ± 0.00^{b}		0.13 ± 0.00
Specific growth rate (%/day)					
50% protein	15.17 ± 0.09^{b}	13.82 ± 0.08^{c}	15.67 ± 0.07^{a}	18.20 ± 0.30	1.49 ± 0.28
55% protein	14.28 ± 0.07^{a}	13.46 ± 0.08^{b}	13.43 ± 0.10^{b}		1.37 ± 0.15
Feed conversion ratio					
50% protein	1.37 ± 0.03^{b}	1.75 ± 0.03^{a}	0.87 ± 0.02^{c}	*	1.33 ± 0.13
55% protein	0.86 ± 0.03^{a}	0.88 ± 0.01^{a}	0.76 ± 0.04^{b}		0.83 ± 0.02

Values are means±S.E.M. of three groups per treatment. Values in the same row with different superscript are significantly different (p<0.05).
* FCR for control was not calculated.

The effect of different protein to energy ratios on weight gain, feed conversion ratio, survival rate, average daily weight gain and specific growth rate are presented in Table 4. Among the formulated diets in Experiment 2, fish larvae fed diet 3 containing 50% protein with 4900 kcal per kg energy had the highest weight gain (3.12±0.00 mg) and highest survival (62.00±2.08%) from other treatments excluding control group. The opposite result was shown in larvae fed diet 5 containing 55% protein with 4700 kcal per kg energy which had the lowest weight gain (1.85±0.00 mg) but still showed significant differences with other treatments.

From the results obtained, it could also be seen that there were significant differences (p<0.05) in larval weight gain, average daily weight gain and specific growth rate. There was significant differences (p<0.05) in feed conversion ratio among the groups. The feed conversion ratio (FCR) ranged from 0.76 to 1.75 for treatment groups. The poorest FCR (1.75±0.03) was recorded in larvae fed diet 2 consisting of 50% protein with 4700 kcal/kg energy. The diet consisting 55% protein with 4900 kcal/kg energy (diet 6) gained the best FCR (0.76±0.04) and it was significantly different with other diets.

As the whole, larvae in the 50% protein showed better weight gain (2.62±0.15 mg) than larvae in the 55% protein group (2.04±0.07 mg). Nevertheless larvae in the 50% protein group showed poorer feed conversion ratio (1.33±0.13) than larvae in 55% protein group which gained better feed conversion ratio (0.83±0.02). The specific growth rates (SGR) determined in all test diets over the experiment showed mean values higher than 3% of body weight per day. The larvae fed diet 3 containing 50% protein and 4900 kcal per kg energy had the highest SGR of 15.67±0.07 % per day, which differs significantly from that with rest of the formulated diets.

Discussion

Results of the trial showed that all diets tested were moderately accepted by the fish. The percentage of survival in all treatments was greater than 40%. Larvae in the control treatment which consumed solely *Artemia* gained the highest weight gain in both Experiments 1 and 2 and were significantly different from that in other treatments. This elucidates that formulated diets might not fully replace live diets for the lemon fin barb hybrid larval species. Larvae still preferred live diets rather than formulated diets as found by Ahammad *et al*. (2009) who discovered that *Barbonymus gonionotus* larvae fed with plankton gained the highest length on day 28 (28.06±0.38 mm) and highest final weight (135.00±3.05 mg).

Tayag (2004) found the optimal protein energy requirement for larvae was 50% protein and 4708 kcal per kg energy. In the current study, lemon fin barb hybrid larvae performed better with diets containing 50% protein and 4900 kcal per kg energy. Larvae in this group achieved the highest weight gain (3.12±0.00 mg) after the control group (fed *Artemia*) and the highest survival (62.00±2.08%). Yousefian *et al.* (2013) observed common carp (*Cyprinus carpio*) larvae fed artificial diets consisting of SFK (a plant ingredient)+ enzyme (lipase, pepsin, trypsin) showed better and significant growth ($p<0.05$) and larvae weight increased from 5.3±0.25 mg to 570.2±0.21 mg, larvae length increased from 5.27±0.21 mm to 30.30±0.25 mm, with better survival rates of more than 70%.

Wolnicki *et al.* (2009) reared larvae of rudd *Scardinius erythrophthalmus* at the age of 4 days post-hatch with a total length (TL) of 5.7 mm and body weight (BW) of 0.9 mg. After 20 days rearing period with the longest period (6 days) of feeding nauplii initially to larvae, then weaned to Aller Futura larvae (AFL), they found larvae achieved TL of 12.4 mm and BW of 17.7 mg. Higher growth was gained by larvae fed on nauplii which achieved TL of 18.9 mm and BW of 68.5 mg. This proved *Artemia* nauplii performed better than AFL for this larval species. A similar situation could be seen in Experiment 1 and 2 in this study as larvae fed on crushed *Artemia* (control group) exhibited best performance in terms of almost all parameters measured.

According to Craig and Helfrich (2009), to create an optimum diet, the ratio of protein to energy must be determined separately for each species. Because fish feed to meet their energy requirements, diets with extra energy levels will result in decreased feed intake and less weight gain. The poorest growth was observed in larvae fed with diet 5 containing 55% protein and 4700 kcal per kg energy which gained the lowest weight gain (1.85±0.00 mg). Moreover this group was significantly different (p<0.05) from other groups in terms of all parameters except for SGR which did not differ significantly from diet 2 and 6. Diet 5 might have been insufficient in energy content and resulted in reduced weight gain as the fish was not able to consume sufficient feed to satisfy energy requirements for growth. When diets consist of extra energy relative to protein content, fish will result in high lipid deposition. As fish should meet their energy requirements, diets with excessive energy levels will result in reduced feed intake and weight gain. Only proper formulated prepared feeds will have a well-balanced energy to protein ratio (Craig and Helfrich, 2009).

Lahnsteiner *et al.* (2012) studied feeding methods for burbot, *Lota lota* larvae in small scale experiments. There was no survival recorded for feeding on artificial microdiets. However, in the current study larvae fed on formulated diet achieved survival rates of more than 40%. Ashraf *et al.* (2009) clarified that striped bass, *Morone saxatilis* larvae fed on microcapsules only limited growth up to 11-25 mg and survival rate did not exceed 25%. This might be because, due to poor digestive system, larvae could not digest microdiets. Ahammad *et al.* (2009) discovered larvae that were fed with plankton only achieved the highest survival rate (92%) followed by larvae fed with plankton and supplementary diets. In the current study, larvae fed with *Artemia* nauplii and artificial diet in this experiment could survive more than 40% until the end of 21 days rearing period. When larvae manage to accept artificial diet with high survival rate, this might alleviate condition where earthen ponds are not prepared or when there is an unavailability of natural feeds (Yousefian *et al.,* 2013). The results of this study indicate that larvae of lemon fin barb hybrid grow well on diets containing 50% protein and 4900 kcal per kg energy with a protein/energy ratio of 97.35 mg protein/ kcal.

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References

Abowei, J.F.N. and Ekubo, A.T., 2011. A review of conventional and unconventional feeds in fish nutrition. *British Journal of Pharmacology and Toxicology***,** 2(**4**), 179-191.

Ahammad, A.K.S., Khan, M.M.R., Hossain, M.A. and Parvez I., 2009. Nursery rearing of Thai sarpunti, *Barbonymus gonionotus* larvae using three different supplementary feeds. *Journal of Bangladesh Agriculture,* 7(**1**), 139-144.

Ahmad, A., Qureshi, T.A., Singh, A.B., 2012. Effect of dietary protein, lipid and carbohydrate contents on the liver composition and enzyme activity of

Cyprinus carpio communis fingerlings. *Journal of Fisheries and Aquaculture*, **4**(**2**), 22-29.

Ashraf, M., Bengtson, D. and Simpson, K.L. 2009. Formulation of semi-purified diets for striped bass, *Morone saxatilis*, Larvae. *Journal of Nutrition*, 8(**10**), 1680-1688.

Bahnasawy, M.H., 2009. Effect of dietary protein levels on growth performance and body composition of monosex Nile tilapia, *Oreochromis niloticus* L. reared in fertilized tanks. *Pakistan Journal of Nutrition*, 8(**5**), 674-678.

Barrows, F.T. and Lellis., W.A. 2006. Effect of diet processing method and ingredient substitution on feed characteristics and survival of larval Walleye, *Sander vitreus*. *Journal of the World Aquaculture Society*, 37(**2**), 154-160.

Catacutan, M.R. and Coloso., R.M., 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 131, 125-133.

Craig, S. and Helfrich., L.A., 2009. Understanding fish nutrition, feeds, and feeding. *Virginia Cooperative Extension*, 420-256.

D'Abramo, L.R., 2002. Challenges in developing successful formulated feed for culture of larval fish and crustaceans. Retrieved from: www.aquatech.com.ve/pdf/A10.pdf. 143-150.

Ghiasvand, Z., Matinfar, A., Valipour, A., Solatani, M. and Kamali, A., 2012. Evaluation of different dietary protein and energy levels on growth performance and body composition of narrow clawed crayfish (*Astacus leptodactylus*). *Iranian Journal of Fisheries Sciences*, 11(**1**), 63-67.

Gomez-Montes, L., Garcia-Esquivel, Z., D'Abramo, L. R., Shimada, A., Vasquez-Pelaez, C. and Viana, M.T., 2003. Effect of dietary protein: Energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. *Aquaculture*, 220, 769-780.

Lahnsteiner, F., Kletzl, M. and Weismann, T., 2012. Rearing of burbot, *Lota lota* (Pisces, toleostei), Larvae with zooplankton and formulated microdiets. *Journal of Agricultural Science*, 4(**9**), 269-277.

Rahman, M.M., 2006. Food web interactions and nutrients dynamics in polyculture ponds. PhD Thesis, Wageningen University, The Netherlands, 1-157.

Shiau, S.Y. and Lan, C.W., 1996. Optimum dieatry protein level and protein to energy ratio for growth of grouper (*Einephelus malabaricus*). *Aquaculture*, 145, 259-266.

Tayag, C.M., 2004. Larval nutrition of silver barb *Barbodes gonionotus* with an emphasis on protein-energy requirements and feeding strategy. Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirements for the Degree of Doctor of Philosophy, 1-5.

Tayag, C.M., Kamarudin, M.S., Saad, C.R. and Aizam, Z.A., 2005. Effect of feeding frequency on the growth and survival of silver barb, *Barbodes* *gonionotus* larvae. *Malaysian Applied Biology Journal*, 34(**2**), 67-73.

Wolnicki, J., Sikorska, J. and Kaminski, R., 2009. Response of larval and juvenile rudd *Scardinius erythrophthalmus* (L.) to different diets under controlled conditions. *Journal of Animal Science*, 7, 331-337.

Yousefian, M., Navazandeh, A., Gharaati, A. and Mahdavi, S., 2013. Investigation of survival, growth and biochemical blood parameters of common carp (*Cyprinus carpio* L.) larvae fed by artificial diets. *International Journal of Plant, Animal and Environmental Sciences*, 175-179.